

## AMENDMENT OF THE SPECIFICATION

Please amend the paragraphs on page 13, lines 6-16, and page 15, line 1, through page 16, line 6, of the specification to read as follows:

Fig. 1 shows the genetic analysis of Dahl S<sup>HSD</sup> rats. (A) Genotyping with contamination-indicative markers (20) corroborates non-genetic contamination of Dahl S<sup>HSD</sup> foundation colony rats. A representative panel is shown for the R80 marker (20) demonstrating non-heterozygosity among Dahl S<sup>HSD</sup> and Dahl R<sup>HSD</sup> foundation colony rats; non-heterozygosity was detected in all contamination-indicative (20) markers (see Table I). The respective sizes of amplified product were: Dahl S = Dahl R with R1041, R138, and R80 markers; Dahl S = Dahl R with R721 GCA, R354. (B) PASA detection of T<sup>1079</sup>/A transversion in Dahl S<sup>HSD</sup> rat genomic DNA corroborates Q276L  $\alpha$ 1 Na,K-ATPase mutation. Comparing two Dahl R (R) and two Dahl S (S) rat genomic DNA samples, PASA analysis using primer-specific for T1079 detects significantly more amplified product in Dahl S rat samples (arrow) compared with Dahl R (R) rat genomic DNA samples at 57°C. Background amplified products could be expected as PASA detects a single base difference. As control, a non-specific marker, Cype (14), was used to indicate relative amounts of genomic DNA in the different samples (arrowhead). Taking the ratio of PASA-product to Cype-amplified product, Dahl S samples exhibit ratios > 1; whereas Dahl R samples exhibit ratios < 1. These results indicate the presence of T<sup>1079</sup> in Dahl S rat genomic DNA corroborating the Q276L  $\alpha$ 1 Na,K-ATPase variant as previously described (8, 9).

Using six microsatellite markers informative for the reported genetic contamination (20), foundation colony Dahl S<sup>HSD</sup> and Dahl R<sup>HSD</sup> rats were checked; no heterozygosity was detected

(Fig. 1 A, ~~Table I~~). Blood pressure phenotypes of foundation colony Dahl S<sup>HSD</sup> and Dahl R<sup>HSD</sup> rats were ascertained using radiotelemetric blood pressure measurements on a high salt (8% NaCl) diet begun at 10 wk of age. Severe salt-sensitive hypertension was detected in male and female Dahl S rats in contrast to salt-resistant normotension in male and female Dahl R rats (~~Table II~~). The data parallel the blood pressure phenotypes reported in the original Dahl S/JR and Dahl R/JR characterization (21). Only after this ascertainment were non-contaminated Dahl S<sup>HSD</sup> and Dahl R<sup>HSD</sup> rats obtained for transgenic experiments begun in 1995. Random testing of transgenic donor female and male Dahl S rats further corroborated absence of genetic contamination.

Additionally, genotyping analysis using a panel of 97 microsatellite markers informative for Dahl S and Dahl R strains and eight markers identical in Dahl S and R strains (14, 22) was done comparing Dahl S<sup>HSD</sup> and Dahl R<sup>HSD</sup> rats used for our experiments, with Dahl S<sup>Rapp</sup> rats obtained by Harlan Sprague Inc. from J. Rapp (21). ~~As seen in Table I,~~ The results show that 103 of 105 markers were identical between Dahl S<sup>HSD</sup> and Dahl S<sup>Rapp</sup> rats; differences were noted at two markers (D1mgh7 and D2mit5); heterozygosity was detected in the Dahl S<sup>Rapp</sup> rats at D2mit13. These results document the non-genetic contamination of Dahl S<sup>HSD</sup> and acceptable polymorphic differences between Dahl S<sup>HSD</sup> and Dahl S<sup>Rapp</sup> due to separate inbreeding over two decades.

Please amend the paragraph on page 16, lines 21-24, and page 18, lines 1-2, of the specification to read as follows:

Based on observations that male and female F1(Dahl S×Dahl R) rats have blood pressures closer to the Dahl R rat strain after 8 wk of high salt (8% NaCl) diet (~~Table II~~), it becomes apparent that SS-EHT in the Dahl S rat model is recessive. Accordingly, a robust transgenic design should involve the transfer of Dahl R wt Q276 1 Na,K-ATPase gene into the Dahl S genetic background, testing its effects on salt-sensitive hypertension phenotype.

Please amend the paragraph on page 22, line 23, through page 23, line 5, of the specification to read as follows:

Blood pressure measurements were then analyzed comparing homozygous male and female transgenic Tg[wt 1]<sub>24</sub> rats with non-transgenic age-matched Dahl S control rats. ~~As seen in Table III, group~~ Group means of 24-h SBP, DBP, and MAP levels in both male and female transgenic Tg[wt $\alpha$ 1]<sub>24</sub> rats were consistently lower than blood pressure levels detected in age-matched control non-transgenic Dahl S rats. Likewise, the levels of increment rise in blood pressure parameters, SBP, DBP, and MAP, after 4 wk of high salt challenge were also significantly lower in both male and female transgenic Tg[wt $\alpha$ 1]<sub>24</sub> rats (~~Table III~~).

Please amend the paragraph on page 25, lines 8-24, of the specification to read as follows:

As shown in Fig. 4, a greater number of magenta PAS-positive abnormal glomeruli are seen in a representative control rat kidney section (Fig. 4 A) compared with a representative transgenic kidney section (Fig. 4 B), indicating less hypertensive renal disease in transgenic rats. This was corroborated by quantitative analysis of renal pathology based on the scoring system described by Raij et al. (18), wherein glomeruli are graded for degree of mesangial thickening and glomerulosclerosis. As shown in Fig. 4, a glomerulus with 25% mesangial thickening and/or glomerulosclerosis is grade I (Fig. 4 D); grade II is 50% pathologic involvement (Fig. 4 E); grade III, 75% involvement (Fig. 4 F); and grade IV, 100% pathologic involvement (Fig. 4 G), in contrast to a normal glomerulus (Fig. 4 C). A total pathology score is calculated with worse severity correlated with higher pathology scores (18). Analysis of renal sections from four control non-transgenic rats (628 total glomeruli scored) compared with five transgenic rat kidney sections (1,213 total glomeruli scored) for severity of mesangial thickening and glomerulosclerosis revealed a 52% decrease in Raij renal pathology score in transgenic rat kidneys compared with control rat kidneys,  $P = 0.0025$  (non-parametric ANOVA) Table IV.

Please amend the paragraph on page 29, lines 1-8, of the specification to read as follows:

As seen in ~~Table V~~ and Fig. 5, the most significant ANOVA results were detected at the  $\alpha 1$  Na,K-ATPase locus (D2mgh11) and at the D2mit14 marker, 2.2 centimorgans (cM) away, for SBP ( $P = 0.00268$ ), DBP ( $P = 0.00920$ ), MAP ( $P = 0.00376$ ). The fact that all three blood

pressure measures provide similar results is in contrast to other F2 cosegregation studies that have detected cosegregation with one blood pressure parameter but not with the others, e.g., locus cosegregation with DBP and pulse pressure, but not with SBP or MAP (23). These results indicate that the  $\alpha 1$  Na,K-ATPase locus meets criterion 4.